Quantity discrimination in the common garden skink and the role of early-life conditions

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## Keywords

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## Abstract

From social behavior to navigating complex environments, quantity discrimination essential for fitness. Animals can use one of two systems to discriminate between different quantities, with variation across taxa. In reptiles, quantity discrimination remained largely unexplored until recently, and the use of one system over the other can depend on the species or the context in which they are tested. Additionally, early life conditions can influence brain development and, in turn, quantity discrimination. For example, early thermal conditions or glucocorticoid concentrations (GCs) — stress-related hormones - when transmitted by the mother, can significantly shape cognition through their effects on brain physiology. Here, we investigated numerical abilities in the common garden skink (*Lampropholis guichenoti*), as well as the effects of elevated prenatal GCs and incubation temperature on numerical discrimination. We assessed numerical abilities using a spontaneous choice test with food as a stimulus. Using a repeated measures design, we subjected lizards to five numerical tests (1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4 prey items), while controlling for continuous variables. Contrary to our predictions, we found no evidence of numerical discrimination in *L. guichenoti*. Furthermore, prenatal conditions did not influence their performance or decision-making. Our findings contribute to the growing body of evidence suggesting that reptiles feeding on live prey may struggle to distinguish between quantities in spontaneous choice tests. Despite these results, further investigation into numerical abilities in live-prey-feeding reptiles and the impact of early conditions on other taxa remains an important area of research.

## Significance statement

Discriminating between quantities can be crucial for individual fitness, yet the ability to distinguish quantities remains poorly understood in reptiles. In this study, we investigated numerical discrimination in *Lampropholis guichenoti*, using a spontaneous choice test with food as the stimulus. Contrary to our predictions, we found no evidence of numerical discrimination in this species. In addition, we explored the impact of early environmental factors, specifically prenatal corticosterone exposure and incubation temperature, on numerical competence. However, we did not observe any effect of these early-life conditions on lizard decision-making or performance. These results open new avenues for exploring the evolution of numerical abilities in reptiles and identifying factors influencing the development of quantity discrimination in this group. Further research should explore alternative methods, such as trained tasks, to better understand the cognitive mechanisms underlying numerical competence in reptiles

## Introduction

The ability to discriminate between quantities is a fundamental cognitive skill widely observed across the animal kingdom ([Vonk and Beran 2012](#ref-vonk2012bears); [Stancher et al. 2015](#ref-stancher2015numerical); [Beran and Parrish 2016](#ref-beran2016capuchin)). Numerical cognition can contribute to social decisions, mate selection, and foraging behaviour, which can be adaptive in various ecological contexts ([Lucon-Xiccato and Dadda 2017](#ref-lucon2017individual); [Nieder 2018](#ref-nieder2018evolution)). For instance, female lions (*Panthera leo*) use numerical information to assess the risk of confronting rival groups ([McComb et al. 1994](#ref-mccomb1994roaring)), male adult mealworms (*Tenebrio molitor*) select sites with scents of more females ([Carazo et al. 2009](#ref-carazo2009quantity)), and red-backed salamanders (*Plethodon cinereus*) prefer tubes containing larger numbers of prey ([Uller et al. 2003](#ref-uller2003salamanders)). Given the adaptive advantages of numerical and quantity discrimination, research has focused extensively on exploring the ability of different species to differentiate between quantities employing different contexts and paradigms.

The main two approaches experimental approaches in the study of quantity discrimination are spontaneous choice tests and training procedures. In spontaneous choice tests, the subjects are presented two groups of biologically relevant stimuli differing in some quantitative traits (number, size, volumen, etc) and the experimenters record if the subjects select or not the most most appropriate quantity (e.g. the group with more conspecifics or the larger amount of food) (Agrillo and Bisazza ([2014](#ref-agrillo2014spontaneous)); Cox and Montrose ([2016](#ref-cox2016quantity))). In training procedures, animals must learn a numerical rule to receive a reward ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous); [Miletto Petrazzini et al. 2018](#ref-miletto2018quantity)). Both methods are believed to be complementary to each other, and both can provide different results even in the same species Miletto Petrazzini et al. ([2018](#ref-miletto2018quantity)). One reason is that spontaneous choice tests assume that one of the options presented is more adequate than the other when this may not always be the case. For example, choosing groups of conspecifics with less individuals could be advantageous when food is scarce but disadvantageous when predators are abundant ([Yee et al. 2013](#ref-yee2013costs)). As such, context and motivation can be important drivers of decision-making that can change the way we measure animals’ abilities to discriminate between quantities ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)).

The other reason is that each method may engage distinct cognitive mechanisms when processing numerical information ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). In this regard, researchers propose that two core systems underlie numerical cognition: the approximate number system (ANS) and the object file system (OFS) ([Hyde 2011](#ref-hyde2011two)). The ANS estimates the approximate magnitude of items within a set, with accuracy decreasing as the numerical difference between sets decreases — a principle known as Weber’s law ([Hyde 2011](#ref-hyde2011two)). In contrast, the OFS allows for the precise tracking of discrete items but is thought to be limited to small quantities, typically up to three or four ([Hyde 2011](#ref-hyde2011two)). The engagement of these systems depends not only on the numerical magnitude but also on contextual factors such as the availability of continuous variables (e.g., area, density, movement) and the nature of the task ([Stancher et al. 2015](#ref-stancher2015numerical)). The presence of such continuous cues typically favors ANS-based processing, while in their absence, the OFS is more likely to be employed ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)).

The other reason is that each method may tap into different cognitive mechanisms ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). In this sense, researchers consider two core systems within the brain that allow for the processing of quantity information: the approximate number system (ANS) and the object file system (OFS) ([Hyde 2011](#ref-hyde2011two)). ANS encodes the approximate magnitudes of items within sets, with accuracy decreasing as the distance between numbers decreases, obeying the called Weber’s law ([Hyde 2011](#ref-hyde2011two)). OFS, on the other hand, allows for the precise tracking of discrete items within sets, and it is thought to be limited to small numbers (typically up to three or four) ([Hyde 2011](#ref-hyde2011two)). However, the use of either system may be dictated, not only by the magnitude of the stimulus, but also by factors such as the availability of continuous information or the context of the task ([Stancher et al. 2015](#ref-stancher2015numerical)). For example, the presence of continuous variables such as area, density, or movement usually entails the use of the ANS, while the OFS would be predominant in the absence of those continuos cues (Agrillo and Bisazza ([2014](#ref-agrillo2014spontaneous))). This often complicates the study of numerosity, as continuous traits must be carefully controlled to ensure the use of the OFS ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). In spontaneous choice tests, for instance, animals may rely on cumulative surface area rather than numerical differences when selecting between two sets of food items ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). To disentangle these cues, researchers often equate the total area of the sets while changing the number of items, forcing individuals to rely on numerical information alone ([Stancher et al. 2015](#ref-stancher2015numerical)).

Quantity discrimination studies on both systems have been traditionally focused on mammals, birds, and fishes despite the influence of numerical cognition on fitness across taxa ([Mehlis et al. 2015](#ref-mehlis2015quantification); [Nieder 2018](#ref-nieder2018evolution)). However, recent studies demonstrate that other vertebrates like amphibians or non-avian reptiles are also competent in numbers ([Uller et al. 2003](#ref-uller2003salamanders); [Stancher et al. 2015](#ref-stancher2015numerical); [Miletto Petrazzini et al. 2017](#ref-miletto2017quantitative)). For example, red-footed turtles (*Chelonoidis carbonaria*) rapidly learned to associate colour with higher volumes of food ([Soldati et al. 2017](#ref-soldati2017long)), Hermann’s tortoises (*Testudo hermanni*) discriminate between large and small food quantities in a spontaneous choice test ([Gazzola et al. 2018](#ref-gazzola2018continuous)), and freshwater turtles (*Trachemys scripta* and *Mauremys sinensis*) rapidly learned a quantity discrimination task ([Lin et al. 2021](#ref-lin2021superior), [2024](#ref-lin2024trained)). The Italian wall lizard (*Podarcis siculus*) failed to discriminate between different numbers of food items in a spontaneous choice test ([Miletto Petrazzini et al. 2017](#ref-miletto2017quantitative)), but succeeded in a trained quantity discrimination task ([Miletto Petrazzini et al. 2018](#ref-miletto2018quantity)). In contrast, Iberian rock lizards (*Iberolacerta cyreni*) spontaneously preferred larger quantities of crickets when presented five versus ten items ([Recio et al. 2021](#ref-recio_prey_2021)). In skinks, Shinglebacks (*Tiliqua rugosa*) exhibited spontaneous quantity discrimination using both discrete and continuous cues ([Szabo et al. 2024](#ref-szabo2024spontaneous)), while Gidgee skinks (*Egernia stokesii*) discriminated between itemst when they differed in number, but not when size was the main quantitative cue ([Szabo et al. 2021](#ref-szabo_spontaneous_2021)). These studies demonstrate that reptiles have cognitive abilities related to quantity discrimination that have been overlooked in the past, and that the use of the ANS versus the OFS can differ between species and in different contexts ([Szabo et al. 2021](#ref-szabo_spontaneous_2021)). However, cognitive performance is not solely dictated by species differences; developmental conditions can also shape an individual’s ability to process numerical information ([Vila Pouca et al. 2019](#ref-vila_pouca_quantity_2019)).

Cognition can be strongly influenced by developmental conditions because the brain is particularly sensitive to environmental inputs in this life stages ([Zhu et al. 2004](#ref-zhu_prenatal_2004)). For instance, the temperature at which embryos develop can affect neuron density or learning abilities in different species of reptiles ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014); [Amiel et al. 2017](#ref-amiel_effects_2017); [Dayananda and Webb 2017](#ref-dayananda_incubation_2017); [Siviter et al. 2017b](#ref-siviter_incubation_2017); [Abayarathna and Webb 2020](#ref-abayarathna_effects_2020)). These thermal effects on cognition may arise directly or indirectly through impacts on parental condition ([Crino et al. 2023](#ref-Crino_2023)). Temperatures outside the optimal range can trigger stress responses, leading to the release of glucocorticoids (GCs) ([Sapolsky et al. 2000](#ref-sapolsky_how_2000)), which may then be transmitted to the offspring ([Crino et al. 2023](#ref-Crino_2023)). Elevated prenatal GCs can, in turn, influence cognition by altering brain function ([Szuran et al. 1994](#ref-szuran_water_1994); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Crino et al. 2014](#ref-crino_corticosterone_2014-learn); [Farrell et al. 2015](#ref-farrell_developmental_2015-learn); [Bebus et al. 2016](#ref-bebus_associative_2016); [Lui et al. 2017](#ref-lui2017chronic)). As such, the interaction between GCs and develpomental temperature must be relevant in shaping reptiles’ cognition. Most studies on the effects of GC exposure or elevated temperatures during development focused on learning and memory, but few studies have investigated how early-life conditions can affect quantity discrimination despite its importance on animals’ lifes (but see [Vila Pouca et al. 2019](#ref-vila_pouca_quantity_2019)). Furthermore, the interactive effects of prenatal GCs and temperature on cognitive abilities remain largely unexplored.

Here, we investigated the use of numerical cues in the common garden skink (*Lampropholis guichenoti*) using a spontaneous choice test where lizards were presented with different number of food items while controlling for continuous variables. In adition, we examined how prenatal corticosterone (CORT) - the main GC in reptiles - and incubation temperature can affect numerical discrimination and decision-making in foraging contexts. To do so, we manipulated CORT concentration (CORT-treated or a sham control) in *L. guichenoti* eggs and incubated them under one of two temperature regimes (Cold - 23 ± 3ºC or Hot - 28 ± 3ºC) in a 2x2 factorial design. Then, we assessed numerical discrimination abilities on five tests that differed in the relative number of food items: 1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4 crickets. Previous studies in other skinks showed that these species can discriminater between different quantities of food using both the ANS and the OFS ([Szabo et al. 2021](#ref-szabo_spontaneous_2021), [2024](#ref-szabo2024spontaneous)). We predicted *L. guichenoti* to be able to discriminate between different number of crickets even when controlling by other continuous variables (i.e. by using the OFS). Furthermore, we predicted lizards’ decisions to be influenced by the early conditions they were subjected to. In this regard, we hypothesized that lizards incubated at higher temperatures would outperform those incubated at lower temperatures in the numerical discrimination task ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014); [Amiel et al. 2017](#ref-amiel_effects_2017); [Vila Pouca et al. 2019](#ref-vila_pouca_quantity_2019)). We predicted that increased CORT levels would impair numerical competence ([Szuran et al. 1994](#ref-szuran_water_1994); [Zhu et al. 2004](#ref-zhu_prenatal_2004)), with more pronounced effects at lower temperatures. We predicted that lizards incubated at higher temperatures would successfully discriminate between quantities even at high ratios (e.g. 3 VS 4), whereas even the lowest ratios (e.g. 1 VS 4) could be challenging for those exposed to CORT or incubated at lower temperatures.

## Methods

#### Animal husbandry

*Breeding colony* – The lizards tested in our experiment came from a breeding colony established in the lab in 2019. The colony consisted of approximately 180 adults of *L. guichenoti* housed in communal containers (41.5 L x 30.5 W x 21 H cm) with six lizards (2 males and 4 females) per enclosure. Enclosures were provided with non-stick matting, shelter, and several small water dishes filled daily, and lizards were fed mid-size crickets (*Acheta domestica*) three days a week. Crickets were dusted with calcium weekly and multivitamins and calcium biweekly. Room temperatures were set to 22-24ºC, but to ensure a temperature gradient, we employed a heat chord and a heat lamp following a 12 h light:12 h dark cycle, keeping the warm side of enclosures at 32-34ºC.

*Egg collection and incubation* – Between mid-October 2023 to the end of February 2024, we placed small boxes (12.5 L x 8.3 W x 5 H cm) containing vermiculite on one side of the communal enclosures (see above) to provide females with a place to lay the eggs. We checked for eggs in the boxes three days a week. Egg length and width were measured with a digital calliper to the nearest mm (± 0.1 mm), while mass was recorded with a digital scale (OHAUS, Model spx123) to the nearest g (± 0.001 g). Eggs were assigned a clutch number and an individual identity. Then we treated eggs with CORT or a vehicle control (see *Manipulating Early Thermal and CORT Environments* details below) and placed them in individual cups (80 mL) with moist vermiculite (12 g water to 4 g vermiculite). We covered the cups with plastic wrap to retain moisture and left the eggs in incubators (LATWIT 2X5D-R1160) programmed to two different thermal regimes (see *Manipulating Early Thermal and CORT Environments* details below) until hatching. Incubators were checked for the presence of hatchlings three times a week.

*Hatchlings* – Immediately after hatching, we measured snout-vent length (SVL) and tail length (TL) with a ruler to the nearest mm (± 0.1 mm), and mass using a digital scale (OHAUS, Model spx123) to the nearest g (± 0.001 g). Hatchlings were then placed in individual enclosures (18.7L x 13.2W x 6.3H cm) provided with non-stick matting and a small water dish. During this period, they were given water daily and received 3-6 small *A. domestica* crickets three times a week. All care otherwise follows similar protocols to adults (see above).

#### Manipulating Early Thermal and CORT Environments

We manipulated CORT concentration in eggs and incubated them under one of two temperature regimes (Cold - 23 ± 3 ºC or Hot - 28 ± 3 ºC) in a 2x2 factorial design ([Fig. 1](#fig-Methods) A). Eggs were allocated to one of two different developmental stress treatments: CORT-treated eggs were topically supplied with 5 µL of a CORT solution (10 pg/mL). Control treatment eggs received an equal volume of 100% Ethanol. Corticosterone treatments were made by dissolving crystalline corticosterone (Sigma, Cat. No. C2505) in 100% ethanol. We selected doses based on previous studies where CORT treatment increased mean yolk CORT levels ~3.7x higher than control eggs in *Lampropholis delicata* ([Crino et al. 2024](#ref-crino2024eggs)). Then, eggs were incubated in one of the two previously mentioned temperature regimes (Cold or Hot) until hatching. These temperatures represent the upper and lower limit of the natural incubation temperatures for this species ([Qualls and Shine 2000](#ref-qualls2000post)).

The number of eggs per clutch assigned to each hormone and temperature treatment was counterbalanced in a partial split-clutch design. At least one egg per clutch to each treatment, and the remaining eggs were randomly assigned to one of the treatments. When we found less than four eggs in a clutch, we assigned each egg randomly to one of the treatments.

#### Numerical discrimination task

Two weeks before starting the numerical discrimination task (see below), lizards were moved to the experimental arenas for acclimatisation. The arenas were individual medium-size (41 L x 29.7 W x 22 H cm) plastic containers provided with a shelter (9 L x 6 W x 1.5 H cm) on one of the sides and a water dish in the center ([Fig. 1](#fig-Methods) B). Arenas were placed on seven different racks located in two experimental rooms. Each rack was associated with its own CCTV system (device model DVR-HP210475), allowing us to record lizards’ behaviour during the experiment. Despite the conditions in the experimental rooms were the same as in the colony room, we counterbalanced the number of lizards per treatment in each rack to control for any potential effects of the room or the position of the lizard in the rack. During acclimatisation and throughout the experiment, lizards were fed with only one cricket daily, dusted with calcium and multivitamins (see protocol below), and water was supplied *ad libitum*. The temperature of both rooms was set to 22-24ºC, but we also provided a temperature gradient (24-32°C) using a heat cord along the center of the arena and heat lamps in a 12 h light: 12 h dark cycle.

The numerical discrimination test consisted of a series of two-choice trials where lizards were presented with different numbers of food items. Each choice was placed on one of the two lateral platforms of a white 3D printed device (see [Fig. 1](#fig-Methods) B, C) and then covered with a transparent Petri dish to prevent the lizard from obtaining the food. We performed five tests that differed in the ratio between both choices of crickets. Specifically, we compared: 1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4 crickets. The food consisted of small frozen crickets (*A. domestica*) that were thawed and dusted with calcium and multivitamins. We decided to use frozen crickets to avoid movement that would affect lizard behaviour ([Cooper et al. 2024](#ref-cooper2024tell)). Lizards were habituated to eating frozen crickets for one month before the experiments.

The crickets used during the experiment were all the same size, but to control for some continuous traits we changed the orientation of the crickets as illustrated in [Fig. 1](#fig-Methods) D. This method was intended to control for length while minimizing increases in convex hull as much as possible (see Stancher et al. ([2015](#ref-stancher2015numerical))). We tested whether the area occupied was similar in all the test by measuring the total length occupied by all the crickets in both choices for half of the animals (see *Supplementary Material - Control of size in both options*), but we did not explicitly control for convex hull. Our results showed that except for the 1 VS 4 and 1 VS 3 tests, the difference in length between both choices was not different from zero. As such, discrimination between both choices in the rest of the tests (2 VS 4, 2 VS 3, 3 VS 4 crickets) would be produced by the OFS, not the ANS ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). In addition, after the experiments, we conducted another set of trials to test whether the orientation of the crickets could affect lizards’ choice, but we found no effect of orientation (see *Supplementary Material - Prey orientation test*).

To habituate lizards to the experimental conditions, frozen crickets were placed on top of one of the platforms and Petri dishes during each feeding for two weeks prior to experimental trials. The side where the food was placed was changed every day to avoid any side bias during the trials. However, to further control for side biases, the higher number of crickets was randomly placed on the right or the left platform. We also counterbalanced the number of individuals per treatment tested in the same type of trial each day.

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| Fig 1— Experimental design. Panel (**A**) shows the early environment manipulation procedures. (**B**) illustrates the arena where the tests were performed, while panel (**C**) indicates the measurements of the platform used for the experiments. Finally, panel (**D**) displays the types of numerical tests used and the orientation of the crickets in each test. |

All tests were done between 1000-1200 hours when the lizards were most active. Each trial was performed daily for five days between the 17th and 22nd of May 2024. We recorded three main variables: i) *Latency*, defined as the time between the start of the test - when the platform was left in the enclosure - until the first interaction with one of the options; ii) First choice of the lizard (*Choice*), recorded as 1 if the lizard interacted first with the larger amount or 0 if not; and iii) *Interest* shown for the higher amount of food (detailed below). We considered that the lizard interacted with the food when it touched the Petri dish with its snout or when the front half of the body was on top of the platform. For five minutes after the first interaction with one of the options, we recorded the time spent interacting with that option for a maximum of five minutes; we did this for both amounts of food independently of what was the first choice. We measured the interest shown for the higher amount of food (*Interest*) by subtracting the time interacting with the option with fewer crickets to the time spent interacting with the larger amount of food. If there was no interaction with one of the options during the test, we considered the time spent as zero for that option. When there was no interaction with any of the choices, both *Latency*, *Choice*, and *Interest* were noted as NA. We also considered Interest as NA in a few cases where the animals managed to lift the dish and eat the prey; Latency and Choice were recorded and employed in the analyses. We excluded from the analyses all the individuals that did not make any choice in three or more tests. All the analyses were performed by PR, who was blind to the treatment of the lizards, but not the type of test or the cricket’s number as the videos were analysed manually.

Motivation plays a key role in animals’ performance in spontaneous choice tests ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous); [Bisazza et al. 2014](#ref-bisazza2014experim)). To ensure that lizards remained motivated to forage, they were provided with only one cricket during both acclimatization and the numerical task (see above). During acclimatization, lizards were fed at the same time each day as the experiments were conducted. In the numerical task, lizards received a cricket immediately after the test and then fasted for more than 20 hours, which we expected would help standardize fasting levels across individuals. To further control for motivation, we excluded from our analyses any trials in which lizards did not interact with any options or did not eat the cricket provided after the test (n = 0 in both cases). Consequently, we do not consider motivation to be a major factor influencing our results (but see Discussion).

#### Statistical analyses

In our analyses, we fitted a multivariate Bayesian multilevel model using the brm function from the *brms* package ([Bürkner 2017](#ref-burkner2017brms)) in R (version 2.8.2) ([R Core Team 2021](#ref-R)). We ran four parallel MCMC chains of 3000 iterations for each model, with a warmup period of 1000 iterations. We checked that all MCMC chains converged (Rhat < 1.2) and were mixing effectively to ensure we had >1000 effective samples from the posterior distribution.

We modelled the three main recorded behaviours: *Latency*, *Choice*, and *Interest* as the response variables. We assumed normal distributions for Latency (log-transformed) and Interest [family = gaussian()]. The error structure for Choice was modeled using a Bernoulli distribution with a logit link function [family = Bernoulli(link = ‘logit’)]. We included test (1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4), hormone (CORT versus Control), incubation temperature (Cold versus Hot), and the three-way interaction as fixed factors. We also included in our predictors sex, and age. We did not find any effect of sex or age on Choice or Interest, but we found an effect on Latency (see Results). Regardless, we included both factors in all models. Age was centered to zero in the model, and we averaged the predicted values for males and females.

We included lizard identity and clutch as random factors. *L. guichenoti* lays up to two clutches per year ([Joss and Minard 1985](#ref-joss1985reproductive)). Since egg collection was done during half of the breeding season, each clutch likely came from a unique mother, and so, clutch identity captures potential maternal effects.

We used the posterior distributions of parameters from these models to test for between treatment differences in the ability to discriminate different numbers of crickets for each of the numerical tests. Because the posterior distributions were not always normally distributed, we report the median as the main measure of central tendency. We used the 95% Highest Posterior Density Intervals (95% HPDI) using the hdi function in bayestestR ([Makowski et al. 2019](#ref-bayestestR)) to test if the contrasts between treatments for all the variables, or the *Interest* per each test was different form zero. Given Choice was Bernoulli, we modified our null hypotheses to test if differed from a probability of 0.5 as there were only two possible choices and if animals were choosing randomly we would predict each choice would have a 50% probability.

#### Ethical note

Subjects were collected from a breeding colony that had been kept in the lab since 2019. All animals in this experiment were provided humane laboratory housing, thermoregulation opportunities, and light and humidity conditions (see above for details). Lizards in the colony received five to seven crickets per individual three times a week while the subjects in the experiment were fed once per day. All lizards were handled minimally to avoid stress. Experimental procedures and laboratory housing complied with Australian law and were approved by the Australian National University Animal Experimentation Ethics Committee (A2022\_33). Upon completion of experimental trials, lizards were kept in their enclosure and will be euthanized following standard humane procedures once they reach the humane endpoint.

## Results

Overall, males showed lower latencies than females (median of the estimated effect of sex = -0.45, 95% HPDI = [-0.89, -0.02]), and older individuals also made decisions faster (mean estimated effect of age = 0.06, 95% HPDI = [0.01, 0.11]; see Table S3 in *Supplementary Material*). However, we did not see any effect of age or sex on *Choice* or *Interest* (see *Supplementary Material*).

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| Fig 2— Estimates of log-latency (Latency), the probability of choosing the larger number of crickets first (Choice), and the estimated interest in the larger number of crickets (Interest) for each of the numerical tests performed across different developmental treatments. The x-axis represents the estimate, and the y-axis is the posterior density of the estimates. The different colours indicate the different treatments. Points and bars represent the median and 95% Higest Density Intervals (95% HPDI) of the estimates, respectively. Vertical dashed lines in Choice and Interest graphs values 0.5 and 0, respectively. Asterisks indicate values significantly different from 0. |

Neither temperature, CORT, or their interaction affected performance in any of the tests, nor were there any clear patterns suggestive of an effect of the developmental environment on numerical discrimination (see Table 1 and [Fig. 2](#fig-results)). Except for Hot-CORT lizards in 1 VS 3 tests or Cold-Control lizards in 2 VS 3 tests, the predicted probability of choosing the larger number of crickets first was not different from 0.5 ([Fig. 2](#fig-results); but also see Table S1 and Table S2 in *Supplementary Material*). Similarly, interest in the larger number of crickets (*Interest*) was not different from 0 in any of the tests ([Fig. 2](#fig-results); but also see Table S1 and Table S2 in *Supplementary Material*).

*Table 1. Effects of temperature, CORT, and their interaction on Latency, Choice, and Interest in each of the numerical discrimination tests. The table shows the contrasts for each predictor (Temperature = [medianHot - medianCold]; Hormone = [medianControl - medianCORT]; and their Interaction = [(medianHot-Control - medianHot-CORT) - (medianCold-Control - medianCold-CORT)]). 95% Higest Density Intervals (95% HPDI) test the hypothesis that contrasts are different from zero.*

|  | | Tests | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | Predictor | 1VS4 | 1VS3 | 2VS4 | 2VS3 | 3VS4 |
| Latency | Hormone | -0.3 [-1.38 , 0.81] | -0.1 [-1.11 , 0.85] | 0 [-0.96 , 0.98] | -0.2 [-1.09 , 0.78] | -0.3 [-1.16 , 0.52] |
| log(latency) | Temperature | -0.3 [-1.44 , 0.73] | -0.2 [-1.22 , 0.74] | -0.4 [-1.35 , 0.61] | -0.4 [-1.35 , 0.51] | -0.1 [-0.9 , 0.8] |
|  | Interaction | -0.8 [-2.01 , 0.36] | -0.5 [-1.71 , 0.64] | -0.5 [-1.69 , 0.74] | -0.4 [-1.56 , 0.81] | 0 [-1.21 , 1.19] |
| Choice | Hormone | 0 [-0.45 , 0.43] | 0 [-0.43 , 0.52] | 0 [-0.34 , 0.41] | 0.1 [-0.37 , 0.44] | 0.1 [-0.26 , 0.47] |
| log(odds) | Temperature | 0.1 [-0.32 , 0.55] | 0.2 [-0.28 , 0.66] | 0 [-0.36 , 0.39] | -0.3 [-0.64 , 0.16] | 0.2 [-0.19 , 0.54] |
|  | Interaction | -0.3 [-0.79 , 0.19] | -0.4 [-0.86 , 0.06] | 0.1 [-0.39 , 0.62] | -0.2 [-0.71 , 0.26] | -0.1 [-0.64 , 0.34] |
| Interest | Hormone | -1 [-57.93 , 56.22] | -11.8 [-84.09 , 63.11] | 1.2 [-62.9 , 62.09] | 3 [-79.32 , 81.26] | 40.3 [-19.96 , 99.89] |
|  | Temperature | -11.6 [-69.84 , 44.56] | 22.4 [-51.77 , 95.92] | -5.3 [-68.05 , 58.67] | -22.6 [-102.97 , 58.19] | 3.6 [-55.27 , 63.55] |
|  | Interaction | 19.1 [-57.25 , 97.64] | -56.5 [-137.29 , 16.36] | 34.9 [-41.2 , 110.83] | -70.1 [-147.39 , 6.76] | -23.6 [-99.35 , 55.79] |

## Discussion

Contrary to our predictions that numerical cues would influence foraging decisions, and that elevated prenatal CORT or cold incubation temperatures would impair numerical discrimination, we found no significant differences across tests or treatments. Our results indicate that lizards did not discriminate between different numbers of crickets, nor did their performance vary under our experimental conditions. Overall, these findings suggest that *L. guichenoti* did not use numerical or other potential quantitative cues for foraging decisions.

#### Foraging context may influence prey number discrimination in reptiles

Our aim in this study was to investigate whether *L. guichenoti* could discriminate between different number of crickets in a spontaenous choice test relying exclusively on the OFS. This question arose from previous research showing that other species within the Scincidae family could use the OFS in spontaneous discrimination tests ([Szabo et al. 2021](#ref-szabo_spontaneous_2021)). To control for non numerical cues like total length and convex hull, we rotated the position of the crickets in the choice with fewer items, creating a situation where total length was similar in both choices ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous); [Stancher et al. 2015](#ref-stancher2015numerical); [Cooper et al. 2024](#ref-cooper2024tell)). This design intended to test whether *L. guichenoti* could rely on numerical information alone when the ANS was not available. However, this manipulation could have made the task more challenging for lizards, potentially affecting their performance. If *L. guichenoti* primarily relies on the ANS for quantity discrimination, this could explain the absence of a numerical preference. Nonetheless, in two of our tests (1 VS 4 and 1 VS 3 crickets) our manipulation was not sufficient to control for total length (see *Supplementary Material - Control of size in both options*). Yet, we still did not find quantity discrimination in *L. guichenoti*. This suggests that the absence of numerical preferences in our study is unlikely to be an artifact of our manipulation, but rather reflects a genuine lack of quantity discrimination in this species, at least in spontaneous choice tests.

In this regard, lack of numerical discrimination in *L. guichenoti* may be due to insectivorous lizards relying on other cues, such as movement or size, for foraging decisions. In studies where reptiles discriminated between different amounts of food, the relevant stimuli involved either vegetables ([Gazzola et al. 2018](#ref-gazzola2018continuous); [Szabo et al. 2021](#ref-szabo_spontaneous_2021)) or large quantities (e.g., 5 vs. 10)([Recio et al. 2021](#ref-recio_prey_2021)). In contrast, when *Podarcis siculus* were presented different numbers of *Musca domestica* larvae in a spontaneous choice test, the lizards failed to discriminate between quantities despite showing quantity discrimination when prey size was the main cue ([Miletto Petrazzini et al. 2017](#ref-miletto2017quantitative)) or when tested using training procedures ([Miletto Petrazzini et al. 2018](#ref-miletto2018quantity)). Notably, the numbers of food items employed in Miletto Petrazzini et al. ([2017](#ref-miletto2017quantitative)) were always fewer than four. Spontaneous choice tests assume that animals should select the most appropriate quantity, which may not necessarily be the largest one ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). This occurrs if the costs of selecting the larger option outweigh the benefits. For example, when presented two groups with different numbers of conspecifics or if prey handling costs are related to larger quantitative traits like size ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). It could be that the perceived handling costs of larger numbers of prey affected lizard response in our study. Nevertheless, in that scenario, we would still expect animals to exhibit a preference, even if for the smaller amount of food. Additionally we would have expected body size to influence lizards choices, as larger animals may be more adept at handling a greater number of prey items. Miletto Petrazzini et al. ([2017](#ref-miletto2017quantitative)) did not report any effect of body size, and age - a proxy for size - did not affect *L. guichenoti*’s *Choice* or *Interest* in our study. The absence of preference in *P. siculus* and *L. guichenoti* suggests that the benefits of selecting a larger number of prey are not different from selecting fewer. For instance, when considering hunting groups of 1 VS 4 or 2 VS 4 crickets, the probability of success might be similar. Furthermore, while one individual handles a prey item within the patch, other conspecifics could capture the rest. As a result, even if diet might not affect quantity discrimination abilities, insectivorous lizards might not have evolved a preference for larger patches of prey, at least with small numbers (see [Recio et al. 2021](#ref-recio_prey_2021)).

#### Foraging behaviour is robust to early environmental experiences

Although the effects of early environment on cognition is widely studied, little attention has been paid to its impacts on numerical discrimination. To the best of our knowledge, there is only one study examining how developmental conditions influence numerical abilities ([Vila Pouca et al. 2019](#ref-vila_pouca_quantity_2019)). Given the potent effects of early environment on cognition and brain development ([Szuran et al. 1994](#ref-szuran_water_1994); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014); [Lui et al. 2017](#ref-lui2017chronic); [Amiel et al. 2017](#ref-amiel_effects_2017)), it is crucial to explore how early environmental conditions shape numerical abilities across taxa. In our experimental design, prenatal treatments did not influence quantity discrimination in *L. guichenoti*. However, since none of the groups showed any kind of quantity discrimination in our tests, we cannot determine whether prenatal conditions affected the lizards’ numerical abilities or not; and we cannot discount the possibility that early environmental factors may affect quantity discrimination under other approaches or contexts.

Nonetheless, our results showed that decision-making was not influenced by CORT elevations or incubation temperature, as latency to make a choice did not vary across prenatal conditions. This suggests that *L. guichenoti* may be robust to these early-life factors, at least in the context of foraging decisions. Previous studies have reported that pine snakes (*Pituophis melanoleucus*) incubated at low temperatures were slower and less proficient in capturing prey ([Burger 1991](#ref-burger_effects_1991)), but cold incubated bearded dragons (*Pogona vitticeps*) were faster at approaching a novel objects at least at a young age ([Siviter et al. 2017a](#ref-siviter_impact_2017)). Maternal transmitted GCs are also known to reduce exploration and neophilia in different taxa ([Koolhaas et al. 1999](#ref-koolhaas1999coping); [Hope et al. 2018](#ref-hope_incubation_2018)). Additionally, both prenatal CORT and incubation temperature can influence metabolic rate (MR), which in turn could affect motivation in food-based learning paradigms ([Noble et al. 2018](#ref-noble_developmental_2018); [Cossin-Sevrin et al. 2022](#ref-cossin2022effect); but see [Trnik et al. 2011](#ref-trnik_persistent_2011); [Crino et al. 2014](#ref-crino_corticosterone_2014-learn)). In our design, we habituated lizards to the test conditions for two weeks before the experiment to minimize neophilia. And to control for motivation, we standardized hunger levels by providing only one cricket per day and fasting lizards for over 20 hours before testing. Additionally, we excluded trials where lizards failed to interact with either option or refused to eat the cricket provided after testing. These measures likely minimized variation in decision latency due to differences in exploration, neophilia, or hunger levels, which could explain why we did not detect an effect of prenatal conditions. Interestingly, we found that males and older lizards made decisions faster, although these factors were not central to our main hypotheses. However, it is possible that sex and aging interact with early-life conditions in shaping behavioral responses. Future research should investigate potential interactions between developmental environment, sex, and age to better understand how these factors influence decision-making in reptiles.

#### Conclusion

Our study was focused on investigating if *L. guichenoti* could use the OFS to disriminate between different number of food items. Aditionally, we were interested in exploring, for the first time, the impact of prenatal conditions on numerical abilities in reptiles. Our findings suggest that *L. guichenoti* does not rely on numerical information for foraging decisions and that their performance and decision making does not vary due to prenatal treatments. These results align with other studies showing that insectivorous reptiles fail in quantity discrimination tasks in spontaneous choice tests. However, it is critical to note that the methodologies employed in this study may not have fully captured the nuances of numerical cognition in *L. guichenoti*. Therefore, we recommend that future studies employ a broader array of tests and paradigms, including training-based tasks, larger sets of stimuli, and other sensory modalities, to more comprehensively assess numerical competence in this and related species. Furthermore, it would be relevant to study the effect of early environment on quantity discrimination to advance in our understanding of nuerosity across taxa.

## Data accessibility

All data, data description, and R code are available in public repository <https://github.com/Pablo-Recio/CORT-Temp_Numerical>.

## Declaration of AI use

We declare Chat GPT was used for questions related to coding and data analyses. All other parts of the manuscript were written by the authors and those parts where AI were used were checked and modified accordingly by the authors.

## Authors’ contributions

PR: conceptualization, methodology, data collection, data curation, formal analysis, writing—original draft, writing—review and editing; DL: data collection, writing—review and editing; OC: conceptualization, writing—review and editing; CF: conceptualization, funding acquisition, writing—review and editing; AP: data collection, writing—review and editing; DN: conceptualization, methodology, funding acquisition, project administration, resources, supervision, writing—review and editing.  
All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

## Conflict of interest declaration

We declare we have no competing interests.

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# Suplementary Material

#### Effects of treatments on numerical discrimination using the estimates and the raw data

*Table S1.- Median and 95% Higest Density Intervals (95% HPDI) of the estimated probability of choosing first the higher amount (Choice) and the estimated interest for the higher amount of food (Interest) per treatment group for each of the numerical tests performed. 95% HPDI test the hypothesis that Choice = 0.5, and Interest = 0, which would indicate a preference towards one of choices.*

|  | | Choice | | Interest | |
| --- | --- | --- | --- | --- | --- |
| Test | Treatment | Median | 95% HPDI | Median | 95% HPDI |
| 1VS4 | Cold-CORT (n = 20) | 0.33 | [0.12 , 0.58] | 8.10 | [-32.66 , 47.59] |
|  |  | 0.47 | [0.24 , 0.72] | -2.45 | [-43.62 , 33.24] |
|  |  | 0.60 | [0.34 , 0.84] | -13.15 | [-53.14 , 27.65] |
|  |  | 0.43 | [0.19 , 0.68] | -4.55 | [-43.02 , 33.76] |
| 1VS3 | Cold-CORT (n = 20) | 0.39 | [0.15 , 0.64] | -19.39 | [-58.47 , 21.02] |
|  |  | 0.65 | [0.4 , 0.86] | -2.65 | [-40.59 , 36.91] |
|  |  | 0.81 | [0.61 , 0.96] | 31.11 | [-7.83 , 70.07] |
|  |  | 0.64 | [0.38 , 0.86] | -8.66 | [-48.91 , 30.31] |
| 2VS4 | Cold-CORT (n = 20) | 0.50 | [0.24 , 0.75] | 12.54 | [-27.51 , 52.52] |
|  |  | 0.48 | [0.23 , 0.71] | -3.62 | [-40.74 , 34.38] |
|  |  | 0.45 | [0.21 , 0.71] | -10.14 | [-48.69 , 29.41] |
|  |  | 0.55 | [0.29 , 0.79] | 8.57 | [-28.9 , 49.12] |
| 2VS3 | Cold-CORT (n = 20) | 0.60 | [0.32 , 0.84] | -9.81 | [-50.74 , 28.81] |
|  |  | 0.76 | [0.53 , 0.93] | 27.75 | [-11.03 , 65.22] |
|  |  | 0.44 | [0.19 , 0.69] | 3.32 | [-37.77 , 41.31] |
|  |  | 0.37 | [0.15 , 0.63] | -29.20 | [-68.05 , 10.62] |
| 3VS4 | Cold-CORT (n = 20) | 0.41 | [0.16 , 0.67] | -30.21 | [-70.83 , 10.18] |
|  |  | 0.59 | [0.35 , 0.83] | 21.83 | [-15.42 , 60.79] |
|  |  | 0.65 | [0.39 , 0.88] | -14.19 | [-56.39 , 25.32] |
|  |  | 0.70 | [0.46 , 0.91] | 14.21 | [-25.9 , 53.62] |

*Table S2.- Performance of each treatment in each of the numerical tests using the raw data. For the variable Latency and Interest, we show the median and the 95% CI. For the varaible Choice, we show the proportion of individuals that chose the higher number of crickets first.*

|  | | | | Tests |  | |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | Treatment | 1 VS 4 | 1 VS 3 | 2 VS 4 | 2 VS 3 | 3 VS 4 |
| Latency | Hot-Control (n = 20) | 595.5 [55.88 , 1593.72] | 721.25 [192.5 , 2074.55] | 622.8 [74.23 , 1447.22] | 666.35 [84.63 , 2311.7] | 742.2 [71.33 , 2344.85] |
|  | Hot-CORT (n = 20) | 1235.7 [257.98 , 4483.92] | 1011.5 [203.12 , 2379.73] | 852.4 [115.38 , 2527.57] | 840.9 [98.2 , 2524.95] | 1360.25 [145.43 , 6131.73] |
|  | Cold-Control (n = 20) | 663.35 [84.93 , 2605.7] | 729.95 [92.8 , 3099.77] | 630.5 [94.1 , 2500.82] | 575 [70.75 , 1679.95] | 491.15 [24.63 , 1915.57] |
|  | Cold-CORT (n = 20) | 880.25 [29.75 , 4557.85] | 760.3 [60.08 , 3493.4] | 703 [30.88 , 3522.77] | 962.15 [35.93 , 4747.52] | 1031.05 [19.88 , 4106.82] |
| Choice | Hot-Control (n = 20) | 8 | 12 | 10 | 7 | 13 |
|  | Hot-CORT (n = 20) | 11 | 15 | 9 | 9 | 11 |
|  | Cold-Control (n = 20) | 10 | 13 | 10 | 15 | 12 |
|  | Cold-CORT (n = 20) | 8 | 9 | 11 | 12 | 9 |
| Interest | Hot-Control (n = 20) | -6.5 [-126.97 , 163.07] | -10.68 [-180.9 , 113.25] | 6.6 [-85.05 , 162.3] | -31.1 [-118.62 , 56.62] | 12.05 [-186.6 , 228.85] |
|  | Hot-CORT (n = 20) | -15.11 [-152.65 , 61.45] | 28.65 [-152.27 , 185.65] | -12.74 [-120.8 , 182] | 1.68 [-108.4 , 120.55] | -15.71 [-148 , 84.4] |
|  | Cold-Control (n = 20) | -2.75 [-141.42 , 204.65] | -3.05 [-152.55 , 179.07] | -3.95 [-142.12 , 104] | 27.25 [-77.38 , 130] | 21.55 [-101 , 164.22] |
|  | Cold-CORT (n = 20) | 12.9 [-188.15 , 195.67] | -14.15 [-203.8 , 123.02] | 17.6 [-105.78 , 186.17] | -4.68 [-119.05 , 79.8] | -24.84 [-182.8 , 74.95] |

#### Model results

*Table S3A.- Summary of the model fitted for loglatency (Latency)*

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| b\_loglatency\_Intercept | 6.47 | 6.47 | 0.31 | 5.96 | 7.00 | 1.00 | 4547.93 | 6871.87 |
| b\_loglatency\_test\_type1VS3 | -0.03 | -0.03 | 0.21 | -0.37 | 0.31 | 1.00 | 5851.20 | 8069.68 |
| b\_loglatency\_test\_type2VS4 | -0.13 | -0.13 | 0.21 | -0.46 | 0.21 | 1.00 | 5607.53 | 8779.16 |
| b\_loglatency\_test\_type2VS3 | -0.02 | -0.02 | 0.21 | -0.36 | 0.33 | 1.00 | 5792.88 | 8099.56 |
| b\_loglatency\_test\_type3VS4 | -0.07 | -0.07 | 0.21 | -0.42 | 0.27 | 1.00 | 5394.76 | 8113.94 |
| b\_loglatency\_tempHot | 0.05 | 0.05 | 0.45 | -0.69 | 0.80 | 1.00 | 4240.55 | 6468.22 |
| b\_loglatency\_cortControl | 0.09 | 0.09 | 0.35 | -0.50 | 0.66 | 1.00 | 3501.90 | 6160.76 |
| b\_loglatency\_sexm | -0.46 | -0.45 | 0.22 | -0.82 | -0.10 | 1.00 | 5456.02 | 7259.40 |
| b\_loglatency\_age | 0.06 | 0.06 | 0.02 | 0.02 | 0.10 | 1.00 | 5242.87 | 6844.75 |
| b\_loglatency\_test\_type1VS3:tempHot | -0.03 | -0.03 | 0.30 | -0.52 | 0.46 | 1.00 | 5246.33 | 7514.61 |
| b\_loglatency\_test\_type2VS4:tempHot | -0.18 | -0.18 | 0.30 | -0.67 | 0.31 | 1.00 | 5432.60 | 8115.85 |
| b\_loglatency\_test\_type2VS3:tempHot | -0.25 | -0.25 | 0.30 | -0.74 | 0.25 | 1.00 | 5517.51 | 7473.31 |
| b\_loglatency\_test\_type3VS4:tempHot | -0.10 | -0.11 | 0.30 | -0.60 | 0.39 | 1.00 | 5247.17 | 7570.16 |
| b\_loglatency\_test\_type1VS3:cortControl | 0.04 | 0.04 | 0.29 | -0.43 | 0.53 | 1.00 | 5376.57 | 7715.18 |
| b\_loglatency\_test\_type2VS4:cortControl | 0.15 | 0.16 | 0.29 | -0.33 | 0.64 | 1.00 | 5492.31 | 8062.19 |
| b\_loglatency\_test\_type2VS3:cortControl | -0.04 | -0.04 | 0.29 | -0.52 | 0.44 | 1.00 | 5459.05 | 7755.73 |
| b\_loglatency\_test\_type3VS4:cortControl | -0.38 | -0.38 | 0.29 | -0.87 | 0.09 | 1.00 | 5264.74 | 8063.37 |
| b\_loglatency\_tempHot:cortControl | -0.78 | -0.78 | 0.49 | -1.58 | 0.03 | 1.00 | 3491.24 | 5908.08 |
| b\_loglatency\_test\_type1VS3:tempHot:cortControl | 0.25 | 0.25 | 0.42 | -0.43 | 0.94 | 1.00 | 5103.28 | 7366.37 |
| b\_loglatency\_test\_type2VS4:tempHot:cortControl | 0.28 | 0.28 | 0.42 | -0.40 | 0.97 | 1.00 | 4860.78 | 7733.09 |
| b\_loglatency\_test\_type2VS3:tempHot:cortControl | 0.37 | 0.37 | 0.42 | -0.32 | 1.05 | 1.00 | 5243.01 | 8532.21 |
| b\_loglatency\_test\_type3VS4:tempHot:cortControl | 0.74 | 0.75 | 0.42 | 0.05 | 1.44 | 1.00 | 5119.54 | 7726.59 |

*Table S3B.- Summary of the model fitted for Choice*

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| b\_choice\_Intercept | -0.68 | -0.68 | 0.58 | -1.64 | 0.26 | 1.00 | 4717.07 | 6912.29 |
| b\_choice\_test\_type1VS3 | 0.24 | 0.24 | 0.69 | -0.89 | 1.38 | 1.00 | 5106.43 | 7093.98 |
| b\_choice\_test\_type2VS4 | 0.71 | 0.70 | 0.69 | -0.42 | 1.86 | 1.00 | 4984.93 | 7514.81 |
| b\_choice\_test\_type2VS3 | 1.12 | 1.10 | 0.73 | -0.06 | 2.35 | 1.00 | 4902.86 | 6926.45 |
| b\_choice\_test\_type3VS4 | 0.33 | 0.32 | 0.70 | -0.82 | 1.48 | 1.00 | 5219.20 | 7158.11 |
| b\_choice\_tempHot | 1.13 | 1.12 | 0.83 | -0.23 | 2.50 | 1.00 | 4283.18 | 6599.41 |
| b\_choice\_cortControl | 0.61 | 0.60 | 0.74 | -0.59 | 1.84 | 1.00 | 3922.51 | 6114.01 |
| b\_choice\_sexm | -0.06 | -0.06 | 0.30 | -0.54 | 0.43 | 1.00 | 14505.46 | 8697.98 |
| b\_choice\_age | -0.04 | -0.04 | 0.03 | -0.09 | 0.01 | 1.00 | 12392.71 | 8820.47 |
| b\_choice\_test\_type1VS3:tempHot | 0.80 | 0.79 | 1.04 | -0.88 | 2.55 | 1.00 | 4972.18 | 6745.87 |
| b\_choice\_test\_type2VS4:tempHot | -1.33 | -1.33 | 1.00 | -2.98 | 0.29 | 1.00 | 4886.89 | 7191.48 |
| b\_choice\_test\_type2VS3:tempHot | -1.77 | -1.77 | 1.04 | -3.49 | -0.09 | 1.00 | 4289.06 | 6024.53 |
| b\_choice\_test\_type3VS4:tempHot | -0.13 | -0.13 | 1.02 | -1.80 | 1.57 | 1.00 | 4538.82 | 7185.57 |
| b\_choice\_test\_type1VS3:cortControl | 0.48 | 0.47 | 0.99 | -1.15 | 2.12 | 1.00 | 4938.94 | 7818.41 |
| b\_choice\_test\_type2VS4:cortControl | -0.70 | -0.69 | 0.98 | -2.32 | 0.90 | 1.00 | 4749.40 | 7672.85 |
| b\_choice\_test\_type2VS3:cortControl | 0.15 | 0.16 | 1.04 | -1.53 | 1.85 | 1.00 | 4980.80 | 8127.50 |
| b\_choice\_test\_type3VS4:cortControl | 0.14 | 0.13 | 1.00 | -1.50 | 1.77 | 1.00 | 4881.82 | 7388.95 |
| b\_choice\_tempHot:cortControl | -1.33 | -1.32 | 1.06 | -3.06 | 0.40 | 1.00 | 3598.45 | 6418.88 |
| b\_choice\_test\_type1VS3:tempHot:cortControl | -0.65 | -0.64 | 1.44 | -3.01 | 1.71 | 1.00 | 4947.05 | 7258.07 |
| b\_choice\_test\_type2VS4:tempHot:cortControl | 1.82 | 1.79 | 1.41 | -0.52 | 4.12 | 1.00 | 4832.87 | 7213.76 |
| b\_choice\_test\_type2VS3:tempHot:cortControl | 0.25 | 0.25 | 1.45 | -2.12 | 2.59 | 1.00 | 4689.39 | 7011.19 |
| b\_choice\_test\_type3VS4:tempHot:cortControl | 0.81 | 0.80 | 1.44 | -1.57 | 3.20 | 1.00 | 4767.29 | 7038.46 |

*Table S3C.- Summary of the model fitted for Interest*

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| b\_comparedinterest\_Intercept | 12.58 | 12.75 | 20.03 | -20.62 | 45.52 | 1.00 | 4226.87 | 6914.84 |
| b\_comparedinterest\_test\_type1VS3 | -27.45 | -27.46 | 24.85 | -68.46 | 13.12 | 1.00 | 5057.66 | 7251.20 |
| b\_comparedinterest\_test\_type2VS4 | 4.45 | 4.37 | 25.04 | -36.11 | 45.40 | 1.00 | 4955.22 | 7144.03 |
| b\_comparedinterest\_test\_type2VS3 | -18.02 | -18.08 | 25.03 | -59.03 | 23.55 | 1.00 | 4608.81 | 7655.14 |
| b\_comparedinterest\_test\_type3VS4 | -38.45 | -38.44 | 25.60 | -80.23 | 3.89 | 1.00 | 4974.12 | 7540.36 |
| b\_comparedinterest\_tempHot | -21.42 | -21.25 | 28.74 | -68.59 | 25.65 | 1.00 | 3724.65 | 6038.13 |
| b\_comparedinterest\_cortControl | -10.87 | -10.78 | 25.67 | -53.25 | 31.15 | 1.00 | 3414.61 | 5708.73 |
| b\_comparedinterest\_sexm | -8.60 | -8.64 | 9.32 | -23.92 | 7.03 | 1.00 | 17607.01 | 8664.01 |
| b\_comparedinterest\_age | -0.68 | -0.67 | 0.97 | -2.25 | 0.91 | 1.00 | 15664.51 | 9250.40 |
| b\_comparedinterest\_test\_type1VS3:tempHot | 71.62 | 71.63 | 35.19 | 13.31 | 129.60 | 1.00 | 4600.90 | 6586.40 |
| b\_comparedinterest\_test\_type2VS4:tempHot | -1.40 | -1.38 | 35.97 | -59.74 | 58.37 | 1.00 | 4594.12 | 6529.68 |
| b\_comparedinterest\_test\_type2VS3:tempHot | 34.51 | 34.43 | 35.89 | -23.35 | 93.63 | 1.00 | 4206.05 | 6855.33 |
| b\_comparedinterest\_test\_type3VS4:tempHot | 37.54 | 37.66 | 36.94 | -22.35 | 97.98 | 1.00 | 4702.14 | 7554.35 |
| b\_comparedinterest\_test\_type1VS3:cortControl | 27.46 | 27.64 | 35.35 | -30.54 | 86.37 | 1.00 | 4866.97 | 7599.14 |
| b\_comparedinterest\_test\_type2VS4:cortControl | -5.20 | -5.27 | 35.16 | -62.72 | 52.41 | 1.00 | 4362.77 | 6797.39 |
| b\_comparedinterest\_test\_type2VS3:cortControl | 48.43 | 48.30 | 35.49 | -9.54 | 106.81 | 1.00 | 4047.11 | 6637.73 |
| b\_comparedinterest\_test\_type3VS4:cortControl | 63.11 | 62.88 | 35.72 | 4.22 | 122.72 | 1.00 | 4618.01 | 7533.53 |
| b\_comparedinterest\_tempHot:cortControl | 19.41 | 19.72 | 36.63 | -40.29 | 79.85 | 1.00 | 3346.75 | 5960.45 |
| b\_comparedinterest\_test\_type1VS3:tempHot:cortControl | -75.56 | -75.33 | 50.52 | -157.46 | 7.56 | 1.00 | 4608.18 | 7070.71 |
| b\_comparedinterest\_test\_type2VS4:tempHot:cortControl | 15.19 | 15.16 | 50.30 | -69.06 | 97.00 | 1.00 | 4217.24 | 6394.78 |
| b\_comparedinterest\_test\_type2VS3:tempHot:cortControl | -89.54 | -88.78 | 50.35 | -173.98 | -6.66 | 1.00 | 3896.04 | 6636.15 |
| b\_comparedinterest\_test\_type3VS4:tempHot:cortControl | -43.38 | -43.71 | 51.44 | -127.24 | 41.39 | 1.00 | 4491.70 | 6562.71 |

*Table S3D.- Summary of the model fitted for other effects*

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| sd\_clutch\_\_loglatency\_Intercept | 0.34 | 0.34 | 0.19 | 0.04 | 0.67 | 1.01 | 716.16 | 2313.13 |
| sd\_lizard\_id\_\_loglatency\_Intercept | 0.81 | 0.81 | 0.10 | 0.64 | 0.97 | 1.00 | 1725.51 | 2777.99 |
| sd\_clutch\_\_choice\_Intercept | 0.26 | 0.23 | 0.18 | 0.02 | 0.60 | 1.00 | 3913.70 | 5803.67 |
| sd\_lizard\_id\_\_choice\_Intercept | 0.59 | 0.60 | 0.25 | 0.13 | 0.99 | 1.00 | 2047.51 | 3110.77 |
| sd\_clutch\_\_comparedinterest\_Intercept | 9.92 | 9.22 | 6.32 | 1.03 | 21.27 | 1.00 | 4065.36 | 6333.93 |
| sd\_lizard\_id\_\_comparedinterest\_Intercept | 9.31 | 8.34 | 6.38 | 0.81 | 20.85 | 1.00 | 3847.09 | 5748.30 |
| sigma\_loglatency | 0.65 | 0.65 | 0.03 | 0.61 | 0.70 | 1.00 | 12611.43 | 8861.89 |
| sigma\_comparedinterest | 78.78 | 78.70 | 3.03 | 73.99 | 83.90 | 1.00 | 15359.76 | 8383.11 |
| lprior | -29.26 | -29.25 | 0.08 | -29.40 | -29.13 | 1.00 | 8343.29 | 9738.65 |
| lp\_\_ | -3440.63 | -3440.02 | 18.68 | -3472.06 | -3410.58 | 1.00 | 2890.39 | 5974.94 |

#### Testing potential side biases

\_Table S4.- Number of individuals per treatment that chose the right (R) or left (L) side in each of the numerical tests. p-value indicates the result of the binomial test comparing the number of choices between sides.

|  | 1 VS 4 | 1 VS 3 | 2 VS 4 | 2 VS 3 | 3 VS 4 |
| --- | --- | --- | --- | --- | --- |
| Control-Cold (n = 20) | R = 12 | L = 8 , p = 0.5 | R = 12 | L = 8 , p = 0.5 | R = 9 | L = 11 , p = 0.82 | R = 12 | L = 8 , p = 0.5 | R = 12 | L = 8 , p = 0.5 |
| Control-Hot (n = 20) | R = 12 | L = 8 , p = 0.5 | R = 5 | L = 15 , p = 0.04 | R = 9 | L = 11 , p = 0.82 | R = 10 | L = 10 , p = 1 | R = 11 | L = 9 , p = 0.82 |
| CORT-Cold (n = 20) | R = 11 | L = 9 , p = 0.82 | R = 11 | L = 9 , p = 0.82 | R = 11 | L = 9 , p = 0.82 | R = 10 | L = 10 , p = 1 | R = 11 | L = 9 , p = 0.82 |
| CORT-Hot (n = 20) | R = 11 | L = 9 , p = 0.82 | R = 9 | L = 11 , p = 0.82 | R = 9 | L = 11 , p = 0.82 | R = 10 | L = 10 , p = 1 | R = 11 | L = 9 , p = 0.82 |

#### Control of size in both options

On the videos of half of the lizards, we estimated the length of both options using the ruler tool on Photoshop 2024. For each chioce in each test, we first calibrated the masure of one centimeter using the length of the bottom of the platform (see Fig below). After calibrating, we measured the length of the crickets at their maximum length, trying to keep the line parallel to the bottom of the platform (see below). The results are shown in Table S4.

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| Fig 3— Calibration of the ruler tool in Photoshop 2024. The bottom of the platform was used to calibrate the ruler tool to measure the length of the crickets. |

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| Fig 4— Measurement of the length of the crickets. The ruler tool was used to measure the length of the crickets at their maximum length. |

*Table S4.- Mean length (cm) of the crickets in each of the numerical tests. The difference in length between the two options is also shown, with the 95% CI.*

| Test | Mean length small (cm) | Mean length large (cm) | Mean differences in length | 95% CI differences in length |
| --- | --- | --- | --- | --- |
| 1 VS 3 | 0.81 | 1.53 | 0.72 | [0.31 , 1.15] |
| 1 VS 4 | 0.81 | 1.86 | 1.05 | [0.61 , 1.61] |
| 2 VS 3 | 1.72 | 1.58 | -0.14 | [-0.61 , 0.32] |
| 2 VS 4 | 1.76 | 1.85 | 0.10 | [-0.23 , 0.56] |
| 3 VS 4 | 1.55 | 1.85 | 0.30 | [-0.19 , 0.89] |

#### Prey orientation test

We performed a choice test to investigate if the orientation of crickets affected the lizards’ choice. We used the same platform and Petri dish as in the numerical discrimination task, but we placed one cricket on each side of the platform, one oriented vertically in respect to the shelter position (see [Fig. 1](#fig-Methods) B) and the other one horizontal to the shelter. We performed a unique trial randomising the position of the orientation of the cricket. We recorded the number of individuals that chose the cricket oriented vertically and horizontally. Results are shown in the table below.

*Table S5.- Number of individuals per treatment that chose the cricket oriented horizontally (Horizontal) or vertically (Vertical) in our control tests. p-value indicates the result of the binomial test comparing the number of choices between both choices.*

|  | Horizontal | Vertical | p-value |
| --- | --- | --- | --- |
| Cold-Control (n = 20) | 8 | 12 | 0.503 |
| Hot-Control (n = 20) | 7 | 13 | 0.263 |
| Cold-CORT (n = 20) | 12 | 8 | 0.503 |
| Hot-CORT (n = 20) | 9 | 11 | 0.824 |

#### Searching for relevant literature

To look for relevant literature about the effects of early environment on numerical discrimination, we searched in the Web of Science and Scopus using the terms on respectively. The queries employed are detailed below. We looked for manuscripts for all years available. The last search was conducted on the 11th of October 2024; the results below refer to this last search.

We found 24 articles on Web of Science and 22 on Scopus, plus one preprint on Scopus. Out of all the documents, only one study was relevant to our search: Vila Pouca et al. ([2019](#ref-vila_pouca_quantity_2019)). That study appeared in both databases and was the only one that explored the effects of early environment on numerical abilities.

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| Fig 5— Search query on Web of Science. |

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| Fig 6— Search query on Scopus. |

#### Checking the model plots

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